Title: A high fat breakfast attenuates the suppression of appetite and acylated ghrelin during exercise at simulated altitude.

Authors names: Jamie Matu,[1] Kevin Deighton,[1] Theocharis Ispoglou,[1] Oliver M Shannon,[1] and Lauren Duckworth[1]

Department and institution: [1]Institute for Sport Physical Activity & Leisure, Leeds Beckett University, Leeds, United Kingdom

Running heading: Changes in appetite after a high fat and a high carbohydrate breakfast at simulated altitude

Corresponding author: J. Matu, Carnegie Research Institute Room 104, Institute for Sport Physical Activity & Leisure, Leeds Beckett University, Leeds, LS6 3QS, United Kingdom (email: J.Matu@leedsbeckett.ac.uk)

Emails: Jamie Matu: J.Matu@leedsbeckett.ac.uk; Kevin Deighton: K.Deighton@leedsbeckett.ac.uk; Theocharis Ispoglou: T.Ispoglou@leedsbeckett.ac.uk; Oliver M Shannon O.Shannon@leedsbeckett.ac.uk; Lauren Duckworth: L.Duckworth@leedsbeckett.ac.uk

Telephone number: +44 (0)113 8123603

Abstract

High-altitude exposure induces a negative energy balance by increasing resting energy expenditure and decreasing energy intake. This diminished energy intake is likely caused by altitude-induced anorexia and can have detrimental effects for those travelling to high-altitude. We aimed to investigate whether altering the macronutrient composition of breakfast could attenuate altitude-induced anorexia and augment energy intake at high-altitude. Twelve healthy men (aged 26 (8) years, body mass index 23.9 (2.7) $kg \cdot m^{-2}$) completed two, 305 minute experimental trials at 4300m simulated altitude (~11.7% O_2). After an overnight fast, participants entered a normobaric hypoxic chamber and rested for one hour, before receiving either a high fat (HF; 60% fat, 25% carbohydrate) or an isocaloric high carbohydrate (HC; 60% carbohydrate, 25% fat) breakfast. One hour after breakfast, participants performed 60 minutes of treadmill walking at 50% of relative $\dot{V}O_{2max}$. An *ad-libitum* buffet meal was consumed 1h 30 minutes after exercise. Appetite perceptions, blood samples and substrate oxidation rates were measured throughout. A significantly higher area under the curve for composite appetite score was observed during exercise in HF (40 (12) $mm \cdot h^{-1}$) compared with HC (30 (17) $mm \cdot h^{-1}$, P=0.036). During exercise, lower insulin concentrations (P=0.013) and elevated acylated ghrelin concentrations (P=0.048) were observed in HF compared with HC. After exercise there was no significant difference in composite appetite score (P=0.356), acylated ghrelin (P=0.229) or insulin (P=0.513) between conditions. Energy intake at the buffet did not significantly differ between conditions (P=0.384). A HF breakfast attenuated appetite suppression during exercise at 4300m simulated altitude, however *ad-libitum* energy intake did not increase.

Key words: Hypoxia; Medium-chain fatty acids; Energy balance; Altitude-induced anorexia

1 Introduction

Appetite suppression has previously been observed during acute exposure to both simulated [1-3] and terrestrial altitude [4]. This effect appears to be maintained during chronic altitude exposures [5, 6] which is associated with significant decreases in energy intake [7, 8], body mass [7-9] and physical performance at altitude [9, 10]. Additionally, resting energy expenditure is suggested to be elevated at altitude [1, 11], which may further stimulate a negative energy balance. Maintaining energy balance is therefore vital for individuals ascending to altitude to maintain body mass and physical capabilities.

Previous research at sea level has identified that acute dietary interventions can alter postprandial gut hormone responses [12, 13] and subsequent energy intake [14, 15]. It is well established that protein is the most satiating macronutrient [16, 17]. Contrastingly, high fat meals have been found to produce the smallest magnitude of postprandial acylated ghrelin suppression and the highest appetite scores, compared with other macronutrients [12, 13, 18, 19]. Ghrelin is a 28 amino acid peptide which is post-translationally modified at its serine 3 residue with medium-chain fatty acids (MCFAs), catalysed by the enzyme Ghrelin-O-Acyl-Transferase (GOAT) [20, 21]. This acylation of ghrelin is necessary for it to bind with the growth hormone secretagogue receptor-1a (GHS-R1a) and exert its orexigenic effects [22]. Furthermore, des-acylated ghrelin has been found to inhibit the orexigenic effects of acylated ghrelin, independently of the GHS-R1a [23]. A growing body of evidence suggests that ingested MCFAs are directly utilised in the acylation of ghrelin, increasing circulating concentrations of acylated ghrelin [24, 25]. This effect can increase appetite and has been found to promote a positive energy balance, preventing weight loss in cachectic patients [26]. In addition, compared with other macronutrients, high fat meals may result in a decreased energy expenditure due to their lower thermic effect [27].

Several circulating hormones have been implicated in the development of altitude-induced anorexia, including glucagon-like peptide-1, peptide YY and pancreatic polypeptide. However, recent studies have identified acylated ghrelin as the strongest mediator of this response based on concomitant decreases of appetite and circulating acylated ghrelin concentrations at altitude [1, 2, 28]. It seems plausible that the ingestion of a high fat meal rich in MCFAs may increase circulating acylated ghrelin concentrations, elevate subjective appetite ratings, augment energy intake and decrease energy expenditure. A combination of these factors over a prolonged period would be beneficial in a high altitude environment by helping to maintain energy balance and body mass.

The purpose of this study was to compare the effects of a high fat breakfast rich in MCFAs and a high carbohydrate breakfast on appetite, gut hormones, energy intake and substrate oxidation. This study represents the first investigation of an intervention attempting to attenuate reductions in appetite at altitude.

2 Methods

2.1 Participants

Twelve healthy men (age 26 (8) years, body mass index 23.9 (2.7) $kg \cdot m^{-2}$, body mass 77 (8.1) kg) volunteered to participate in this study. Informed consent was obtained from all individual participants included in the study. All participants were non-smokers, non-diabetic, normotensive, free from food allergies and were not taking any regular medication. None of the participants had travelled to an altitude >1500m for the previous three months and were currently residing at an altitude of <500m. All study protocols received institutional ethics approval and were performed in accordance with the Declaration of Helsinki.

2.2 Experimental Design

Participants attended the laboratory on four separate occasions. The first visit included screening, anthropometry, a food preferences assessment and a test for sickle cell trait. Sickle cell trait was an exclusion criteria due to complications that may occur in hypoxia, for example splenic infarction [29]. During the second visit participants completed an incremental exercise test at 4300m simulated altitude, to determine a relative treadmill walking speed for subsequent experimental trials, as previously described [1]. In a randomised and counter-balanced fashion, two experimental trials were conducted ≥48h following the incremental exercise test and were separated by ≥7 days. Participants were not informed by the researcher which breakfast they were provided with before or during each trial. During the experimental trials participants consumed either a high fat (HF) or a high carbohydrate (HC) breakfast. Target FiO_2 was adjusted on the morning of each testing day using the following equation: FiO2 = PiO2 / (PB - 47); where PB is barometric pressure in mmHg and 47 mmHg is the vapour pressure of water at 37 °C [30, 31]. Simulated PiO_2 was 83 mmHg (FiO_2 ~11.7%). Temperature and humidity were maintained at 20 °C and 50% for all tests, respectively.

2.3 Experimental trials

Participants recorded their dietary intake on the day prior to their first experimental trial and repeated the quantity and timing of this intake before their second trial. On the day preceding each experimental trial participants also refrained from alcohol, caffeine and strenuous exercise, and consumed a standardised evening meal (4338 kJ, 57% carbohydrate, 28% fat, 15% protein) between 7pm and 8pm. This meal contained: fusilli pasta, pasta sauce, cheddar cheese, milk, and jelly beans and was consumed to minimise the possibility of a 'second meal effect' confounding the outcome measures [32, 33]. Compliance to pre-experimental controls was verbally confirmed with each participant on the morning of each trial. On the day of testing, participants arrived at the laboratory and entered the hypoxic chamber at 8am. At 1h participants were given 15 minutes to consume either a HF (60% fat, 25% carbohydrate, 15% protein) or a HC (60% carbohydrate, 25% fat, 15% protein) breakfast (Table 1). Both breakfasts consisted of cooked porridge served in an oversized bowl with a separate drink of 216mL. Participants remained rested (e.g. reading or watching DVDs; material strongly related to the aims of the study was not permitted) throughout both trials with the exclusion of the exercise period. At 2h 15 minutes a 1h exercise protocol was completed in which participants walked on a treadmill at 50% of relative $\dot{V}O_{2max}$ at a 10% gradient whilst

carrying a 10kg backpack, to mimic the demands of high altitude trekking [34]. Participants then remained rested until the end of the trial at 5h 05 minutes. Rating of perceived exertion (RPE) was measured at 15 minute intervals during exercise [35]. Acute mountain sickness (AMS) was measured every 30 minutes via the Lake Louise Score (LLS) [36] and used to diagnose mild AMS (LLS ≥3 in the presence of a headache) and severe AMS (LLS ≥6 in the presence of a headache).

2.4 Appetite and palatability perceptions

Subjective appetite perceptions were measured at baseline and every 30 minutes throughout each experimental trial, with the exclusion of the 15 minute interval for breakfast consumption. During exercise, subjective appetite perceptions were measured whilst maintaining walking on the treadmill. Validated visual analogue scales (VAS) were used to assess appetite and palatability perceptions [37]. A composite appetite score (CAS) was calculated after inverting the values for fullness and satisfaction [38]. A higher CAS is associated with greater appetite sensations and thus a stronger motivation to eat.

2.5 *Ad-libitum* meal

At 4h 45 minutes participants were given 20 minute access to a cold *ad-libitum* meal that was presented identically between trials, and consisted of: three types of cereal, semi-skimmed milk, orange juice, white bread, brown bread, cheese, ham, tuna, bananas, apples, oranges, crisps, butter, margarine, mayonnaise, cereal bars, chocolate bars, cookies, muffins and chocolate rolls. All foods were provided in excess of expected consumption and participants were informed to 'eat until comfortably full'. Participants were also made aware that additional quantities of each food item were available if desired. Meals were consumed in isolation, inside the hypoxic chamber and behind a privacy screen to minimise any effects of social influence on food intake. Energy intake was calculated by weighing foods before and after consumption (to the nearest 0.1g), and with reference to the manufacturers nutritional information. This *ad-libitum* meal allowed for macronutrient preferences to be assessed.

2.6 Online gas analysis

Expired gas analysis was conducted throughout trials using an online gas analyser (Metalyser® 3B, Cortex, Leipzig, Germany), as previously described [1]. Substrate oxidation was estimated using equations for rest [39] and exercise [40]. Substrate oxidation rates were then used to estimate energy expenditure at rest and during exercise.

2.7 Blood sampling

All venous blood samples were obtained from an antecubital vein using a 20-gauge cannula (Introcan Safety; B Braun, Sheffield, UK). The first blood sample was collected >10 minutes after the cannulation procedure to minimise any effect of vagus nerve stimulation on measured blood analytes [41]. Further samples were drawn at

1h, 2h 15 minutes, 3h 15 minutes, 4h and 4h 45 minutes. From each venous sample a microcuvette and a heparinised micro haematocrit tube were used to collect 10 μL and ~45 μL of whole blood, respectively, for the measurement of haemoglobin and haematocrit concentrations. These data were used to estimate plasma volume changes over time [42]. To minimise the effect of any postural changes in plasma volume all blood samples were collected whilst the participant was seated [43]. At each time point samples were collected into one 4.9 mL and one 9 mL pre-cooled EDTA monovette (Sarstedt, Leicester, UK). The 9 mL tube was used for the determination of plasma concentrations of insulin, glucose, lactate, non-esterified fatty acids (NEFA) and triglycerides. The 4.9 mL tube was used for the determination of plasma concentrations of acylated and des-acylated ghrelin. To prevent the degradation of acylated ghrelin, 4.9 mL tubes were pre-treated on the morning of each experimental trial, as previously described [1, 44]. Immediately after filling, both tubes were spun at 1500 x *g* for 10 minutes in a centrifuge (CompactStar CS4, VWR). Plasma from the 9 mL tube was transferred into cryovials and 1 mL of the plasma from the 4.9 mL tube was mixed with 100 μL of 1M hydrochloric acid. This solution was then spun at 1500 x *g* for five minutes before the supernatant was then transferred into cryovials. All cryovials were then immediately frozen at -20 °C before being transferred to -80°C and stored until analysis.

2.8 Blood Analysis

Commercially available enzyme-linked immunosorbent assays were used to determine plasma concentrations of acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France), des-acylated ghrelin (SPI BIO, Montigny Le Bretonneux, France) and insulin (IBL, Hamburg, Germany). To eliminate inter-assay variation, all samples from each participant were assayed on the same plate. Photometic analysis was utilised to measure glucose, lactate, NEFA and triglycerides using reagents from Instrumentation Laboratory (Lexington, MA), Randox Laboratories (Crumlin, UK), Wako Chemicals (Dusseldorf, Germany) and Instrumentation Laboratory (Lexington, MA), respectively. The within batch coefficients of variation were as follows: acylated ghrelin 2.4%, des-acylated ghrelin 4.1%, insulin 5.7%, glucose 3.2%, lactate, 2.8%, NEFA 2.8% and triglycerides 3.7%. Total ghrelin was computed via the addition of acylated and des-acylated ghrelin concentrations.

2.9 Statistical analysis

Data are expressed as mean (SD) in text and tables and mean (SE) in figures to avoid distortion of the graphs. All data were analysed using IBM SPSS statistics (v22.0 for Windows; SPSS, Chicago, IL). Area under the curve (AUC) was calculated using the trapezoid method for appetite perceptions and blood parameters. The four AUC periods were defined as: pre-prandial (the 1h before breakfast), postprandial (the 1h after breakfast), exercise (the 1h exercise period), and post-exercise (the 1hr 30 minutes post-exercise). Paired t-tests were used to assess differences in RPE, palatability ratings and energy intake. Two-way repeated measures analysis of variance (ANOVA) was used to assess condition, time, and condition*time based differences in AUC values for appetite perceptions, LLS, blood analyte concentrations, substrate oxidation and energy expenditure. Where significant effects were found, post-hoc analysis was performed using paired *t* tests. . Analysis of covariance (ANCOVA) was performed on appetite perceptions, blood analyte concentrations and energy intake using LLS as a covariate. The interpretation of the findings was unchanged when accounting for LLS as a covariate, and thus the original

data are presented. Effect sizes are presented as Cohen's *d* and interpreted as ≤ 0.2 trivial, >0.2 small, >0.6 moderate, >1.2 large, >2 very large and >4 extremely large [45]. Interpretation of all blood analytes was unchanged when plasma volume changes were accounted for, thus the original data is presented. The sample size used was deemed sufficient to detect significant differences in CAS, acylated ghrelin and energy intake between conditions. Based on effect sizes calculated from previous work in our laboratory[1], and an alpha value of 5%, a sample size of 12 participants would generate a power >95% for these three variables. Calculations were performed using G*power [46].

3 Results

3.1 Exercise responses and acute mountain sickness

Maximal oxygen uptake at 4300m was 39.2 (5.1) $mL \cdot kg \cdot min^{-1}$ and walking speed was 2.8 (0.6) $km \cdot h^{-1}$ during the experimental trials. There was no difference in RPE between the HF (12 (2)) and the HC (12 (2), P = 0.467, *d* = 0.08) conditions during exercise. Mild AMS manifested in four and six participants in the HF and HC conditions, respectively. Severe AMS occurred in one and two participants in the HF and HC conditions, respectively. Two-way repeated measures ANOVA revealed a significant effect of time (P = 0.009) on LLS, however no effect of condition (P = 0.313) or condition*time (P = 0.318) was observed. Mean LLS across the entire trial was 1 (2) during the HF and 1 (1) during in HC condition.

3.2 Appetite and palatability perceptions

Two-way repeated measures ANOVA revealed a significant effect of time (P <0.001) and condition*time (P = 0.026), but not condition (P = 0.223) on CAS. Post-hoc analysis revealed at baseline and during the pre-prandial period there were no significant differences in CAS between the HF and HC conditions (all $P \geq 0.218$, all $d \leq 0.31$). During exercise AUC for CAS was significantly higher in HF (40 (12) $mm \cdot h^{-1}$) compared with HC (30 (17) $mm \cdot h^{-1}$, P = 0.036, *d* = 0.63). During the post-exercise period there was no significant difference in AUC for CAS between conditions (P = 0.356, *d* = 0.26) (Figure 1). Two-way ANOVA revealed no significant effects of condition or condition*time for hunger ($P \geq 0.163$), satisfaction ($P \geq 0.288$) or fullness ($P \geq 0.102$). A significant effect of condition*time was observed for prospective food consumption (P = 0.001). Post-hoc analysis revealed significantly higher prospective food consumption in HF, compared with HC, during the post-prandial (P = 0.019, *d* = 0.92) and exercise (P = 0.016, *d* = 0.88) periods. There was no difference observed for appeal (P = 0.319, *d* = 0.29), smell (P = 0.507, *d* = 0.19), taste (P = 0.843, *d* = 0.06), aftertaste (P = 0.208, *d* = 0.33) and palatability (P = 0.768, *d* = 0.09) of the breakfast between conditions (Supplementary Table 1).

3.3 Energy intake

Mean energy intake at the *ad-libitum* meal was not different after the HF breakfast (5589 (2076) kJ) compared with the HC breakfast (6086 (2235) kJ, P = 0.384, *d* = 0.23). In addition, there were no differences in the absolute or relative consumption of carbohydrate (both $P \geq 0.731$, $d \leq 0.08$), fat (both $P \geq 0.348$, $d \leq 0.27$) or protein (both $P \geq 0.260$, $d \leq 0.31$) (Supplementary Table 2).

3.4 Substrate oxidation and energy expenditure

Two-way repeated measures ANOVA revealed a significant effect of time, condition and condition*time for relative (all $P \leq 0.038$) and absolute (all $P \leq 0.003$) carbohydrate oxidation. Post-hoc analysis revealed that during the pre-prandial period, there were no significant differences in relative or absolute carbohydrate and fat oxidation between conditions (all $P \geq 0.105$, all $d \leq 0.17$). During the postprandial period, exercise and the post-exercise period both relative (all $P \leq 0.012$, $d \geq 0.75$) and absolute (all $P \leq 0.009$, all $d \geq 0.70$) carbohydrate oxidation were significantly higher in HC, compared with HF. A significant effect of time (P < 0.001) and condition*time (P =

0.003) was observed for absolute fat oxidation, however no effect of condition was revealed (P = 0.111). Post-hoc analysis revealed absolute fat oxidation was not significantly different between conditions in the postprandial or the post-exercise period (both P ≥ 0.133, $d \leq 0.26$). However, during exercise absolute fat oxidation was significantly higher after the HF breakfast compared with the HC breakfast (P = 0.014, $d = 0.76$) (Table 2).

Two-way repeated measures ANOVA revealed a significant effect of time (P < 0.001) on energy expenditure but not condition*time (P = 0.617). There was however a tendency towards an effect of condition (P = 0.060) on energy expenditure, with lower values observed for total energy expenditure across the entire trial in HF (3635 (561) kJ) compared with HC (3848 (491) kJ.

3.5 Blood parameters

There were no differences between trials for the concentrations of any blood analyte at baseline (all P ≥ 0.137, all $d \leq 0.18$).

There was a significant main effect of time (P = 0.029) and condition*time (P = 0.002), but not condition (P = 0.100) on acylated ghrelin concentrations. Post-hoc analysis revealed that during the postprandial period AUC for acylated ghrelin tended to be higher after the HF breakfast compared with the HC breakfast (P = 0.069, $d = 0.15$) (Figure 2A). During exercise AUC for acylated ghrelin was significantly higher after the HF breakfast (151.9 (180.2) $pg \cdot mL^{-1} \cdot h^{-1}$) compared with the HC breakfast (100.6 (106.1) $pg \cdot mL^{-1} \cdot h^{-1}$, P = 0.048, $d = 0.35$). During the post-exercise period, AUC for acylated ghrelin was not significantly different between conditions (P = 0.153, $d = 0.14$). There was no effect of time (P = 0.857), condition (P = 0.219) or condition*time (P = 0.605) for des-acylated ghrelin concentrations (Figure 2B). Furthermore, there was a tendency for a main effect of condition (P = 0.052), time (P = 0.079) and condition*time (P = 0.089) for total ghrelin concentrations (Figure 2C).

There was a main effect of time (P = 0.005) and condition*time (0.039), but not condition (P = 0.494) for glucose concentrations. Post-hoc analysis revealed that glucose concentrations tended to be higher and were significantly higher during the postprandial (P = 0.094, $d = 0.25$) and exercise (P = 0.033, $d = 0.37$) periods in HC compared with HF. There was no difference in glucose concentrations between conditions in the post-exercise period (P = 0.199, $d = 0.32$) (Figure 3A). There was a main effect of time (P < 0.001) and condition*time (P < 0.001), and a tendency for condition (P = 0.053) for insulin concentrations. Post-hoc analysis revealed that during the postprandial period AUC for insulin was significantly lower in HF (24.9 (16.1) $\mu lU \cdot mL^{-1} \cdot h^{-1}$) compared with HC (34.3 (14.2) $\mu lU \cdot mL^{-1} \cdot h^{-1}$, P = 0.003, $d = 0.62$). During exercise AUC for insulin was also lower in HF (27.0 (15.9) $\mu lU \cdot mL^{-1} \cdot h^{-1}$) compared with HC (39.5 (15.0) $\mu lU \cdot mL^{-1} \cdot h^{-1}$, P = 0.013, $d = 0.81$). There was no difference in AUC for insulin between conditions in the post-exercise period (P = 0.513, $d = 0.11$) (Figure 3B).

There was a main effect of time (P < 0.001), condition (P = 0.002) and condition*time (P = 0.005) for lactate concentrations. Post-hoc analysis revealed that lactate concentrations were significantly lower in the postprandial, exercise and post-exercise periods (all P ≤ 0.011, all $d \geq 0.62$) in HF compared with HC (Figure 3C). There was a main effect of condition*time (P = 0.002), a tendency for condition (P = 0.086) and no effect of time (P = 0.235) on NEFA concentrations. Post-hoc analysis revealed that during the postprandial period there was no

difference between conditions for concentrations of NEFA (P = 0.553, d = 0.20). However during the exercise and post-exercise periods, concentrations of NEFA were significantly higher in HF compared with HC (both P ≤ 0.047, $d \geq 0.97$) (Figure 4A). There was a main effect of time (P < 0.001) and condition*time (P = 0.015), and a trend for an effect of condition (P = 0.052) on triglyceride concentrations. Post-hoc analysis revealed that during the postprandial period there was a tendency for higher triglycerides concentrations in HF compared with HC (P = 0.078, d = 0.73). During the exercise and post-exercise periods triglyceride concentrations were significantly higher in HF compared with HC (both P ≤ 0.049, $d \geq 0.81$) (Figure 4B).

4 Discussion

This study investigated the effects of a HF and a HC breakfast on changes in appetite perceptions, gut hormones, energy intake and substrate oxidation during a 5h exposure to 4300m simulated altitude. The primary finding of this investigation is that consumption of a HF breakfast, compared with a HC breakfast, resulted in significantly higher CAS and acylated ghrelin concentrations during a subsequent exercise bout. However, this effect was transient and thus did not alter *ad-libitum* energy intake 1hr 30 minutes after exercise. In addition, absolute and relative carbohydrate oxidation was significantly lower in all periods after the HF breakfast compared with the HC breakfast. This effect produced a trend for a lower total energy expenditure in HF compared with HC.

Although AUC for CAS was 31% higher and AUC for acylated ghrelin was 51% higher during exercise in HF compared with HC, no differences were observed in subsequent energy intake. This was likely due to the 1hr 30 minute time period between exercise and *ad-libitum* feeding, in which appetite perceptions and acylated ghrelin concentrations converged between conditions. It seems feasible that a difference in energy intake may have been observed if *ad-libitum* feeding was administered immediately after exercise and future research is warranted in this respect. This future research could hold ecologically validity, as trekking at terrestrial altitude is often followed immediately by a meal, e.g. a stop for lunch. Furthermore, the increased appetite in the present study during exercise in HF may have increased *ad-libitum* feeding during exercise, had foods been made available. This could increase energy intake at terrestrial altitude as snacks are usually available during trekking. The appetite responses in the present study corroborate the findings of similar investigations at sea level. Monteleone, Bencivenga [47] observed that a 77% carbohydrate meal suppressed hunger to a significantly greater extent than a 75% fat meal. Furthermore, previous data has suggested that a high carbohydrate meal induces a greater decrease in postprandial ghrelin concentrations than an isocaloric high fat meal [12, 18, 47]. Previous research shows that this larger postprandial decrease in appetite and plasma ghrelin concentrations, begins to manifest approximately 60 minutes after food ingestion [12, 18, 47], which coincided with the start of exercise in the present study. Therefore, it is possible that the observed differences in appetite and plasma acylated ghrelin concentrations during exercise may be attributable to nutrient transit through the gastrointestinal tract over time, rather than being induced by exercise.

The findings of the present study suggest a possible role of insulin in postprandial appetite suppression at altitude. Insulin has been shown to suppress appetite and food intake via signalling in the hypothalamus [48] and it seems feasible that the larger insulin response following the HC breakfast in the present study may have contributed to the significantly lower appetite perceptions compared with HF. In addition, the higher postprandial insulin concentration in the HC condition could have influenced appetite indirectly by contributing to the larger suppression of acylated ghrelin, compared with the HF condition [49-51]. Without dietary intervention, acylated ghrelin appears to be more strongly associated with altitude-induced anorexia than circulating insulin concentrations [1]. Subsequently it seems reasonable to suggest that changes in CAS in the present study may have been mediated predominantly by the acylated ghrelin responses, as opposed to changes in insulin concentrations. This is the first study to directly influence acylated ghrelin concentrations at simulated altitude via dietary intervention, and this elevation of acylated ghrelin concentrations during exercise resulted in a simultaneous elevation of CAS. This strengthens the concept of a causal relationship between circulating acylated ghrelin concentrations and subjective appetite responses at altitude. This speculation is supported at sea level as

studies have found ghrelin infusion to decrease satiety [52], increase hunger [53-55] and increase energy intake [55, 56] in a variety of populations.

The high-fat breakfast provided within the current study was rich in coconut oil, a foodstuff known for high concentrations of MCFAs [57]. It seems plausible that the significantly higher acylated ghrelin concentrations after the HF breakfast may be due to an increased availability of MCFAs as a substrate for GOAT [24], which is supported by the elevated NEFA and triglyceride concentrations in the present study. This speculation is corroborated by evidence that ingested MCFAs are directly utilised in the acyl modification of ghrelin in rodents [24]. Substantiating this, others have found MCFA ingestion increases circulating acylated ghrelin concentrations in ruminants [58], piglets [59] and cachectic patients [26]. Additionally, following a meal, neural signals are produced from the gastrointestinal tract which represent direct post-ingestive satiety signals, outlined in the satiety cascade [60]. It may be possible that circulating glucose elicits more potent satiating neural signals compared with circulating free fatty acids at altitude.

Entire trial energy expenditure tended to be lower after the HF breakfast compared with the HC breakfast. This phenomenon would be beneficial in minimising an altitude-induced negative energy balance, potentially aiding the maintenance of body composition at altitude, were it to persist. The thermic effect of food is reported to be 0–3% and 5–10% of the caloric content of the fat and carbohydrate administered [27], supporting the lower energy expenditure after the HF breakfast, compared with the HC breakfast. Interestingly we found that, whilst resting, absolute fat oxidation rate remained unchanged between conditions and absolute carbohydrate oxidation was lower after the HF breakfast, explaining the lower energy expenditure in the HF condition. The higher absolute carbohydrate oxidation observed after the HC breakfast aligns with the simultaneously higher lactate concentrations, which substantiates previous data at sea level [61]. This is likely the result of an increased glycolytic flux in the HC condition producing a higher rate of lactate production, and thus plasma lactate concentrations. Recent research has demonstrated an increased reliance on fat oxidation during acute exposure to altitude compared with a matched sea level condition in fed individuals at rest [1] and during exercise [1, 62]. The current study demonstrated that, although reliance on fat as a substrate was already high due to acute hypoxic exposure, feeding with a HF breakfast further increased relative reliance on fat up to 74.6 (12.5)% in the postprandial stage.

Despite the novel findings observed in the present study, some notable limitations must be acknowledged. Firstly, the hypoxic exposure was relatively short and it is possible that acylated ghrelin and CAS may respond differently over a longer period of time. It seems feasible that the consumption of additional high fat meals during more prolonged exposure may produce additional smaller magnitudes of postprandial acylated ghrelin suppression compared with high carbohydrate meals and this area warrants further research. In addition, tightly controlled laboratory studies of this nature are valuable to gain mechanistic understanding and provide a proof of concept but these findings require application in further field studies to assess the effects of high fat feeding when combined with the effects of trekking, gradual ascent and other environmental stimuli (e.g. cold exposure) which occur during real life ascent to high-altitude. It is not possible to attribute the findings of the present study to high fat feeding, or MCFAs *per se*. In order to make this distinction a control high-fat breakfast would be necessary containing minimal amounts of MCFAs, this is a potential area for further research. Additionally, without a sea level control group in the present study it is not possible to state that an altitude related

suppression of appetite occurred. However, in our laboratory we have previously observed significant altitude-induced appetite, acylated ghrelin and energy intake suppression using an extremely similar population and protocol at the same altitude [1]. Therefore it seems likely that participants were suffering appetite suppression as a result of altitude exposure. Finally, human energy balance is regulated by a complex multifaceted system. Although the current study shows an augmentation of appetite after consuming a high fat breakfast, it is overly simplistic to attribute all of the findings to a single gut hormone. Further research is needed to provide a full mechanistic explanation of these findings. In conclusion, the consumption of a high fat breakfast at 4300m simulated altitude attenuated the suppression of CAS and acylated ghrelin during subsequent exercise. However, this was transient and had no effect on energy intake at an *ad-libitum* meal provided 1hr 30 minutes after exercise. In addition, high fat feeding resulted in a lower energy expenditure during the five hour trial in comparison with high carbohydrate feeding. It seems plausible that a combination of these factors would help to maintain energy balance at altitude, however further research would be beneficial to establish whether energy intake can be augmented at altitude.

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Table 1. Characteristics of the breakfasts

Meal	Porridge ingredients	Drink	Total energy and macronutrient composition
High fat breakfast	43 g rolled oats (598 kJ, 26 g carbohydrate, 3 g fat, 4 g protein) 233 mL whole milk (645 kJ, 10 g carbohydrate, 9 g fat, 8 g protein) 26 g coconut oil (961 kJ, 1 g carbohydrate, 25 g fat, 0 g protein) 7 g unflavoured whey (125 kJ, 0 g carbohydrate, 1 g fat, 6 g protein)	216 mL whole milk (597 kJ, 10 g carbohydrate, 9 g fat, 7 g protein)	2927 kJ, 47 g (25%) carbohydrate, 47 g (60%) fat, 25 g (15%) protein
High carbohydrate breakfast	81 g rolled oats (1122 kJ, 49 g carbohydrate, 6 g fat, 8 g protein) 437 mL semi-skimmed milk (868 kJ, 20 g carbohydrate, 8 g fat, 15 g protein) 25 g maltodextrin (369 kJ, 24 g carbohydrate, 0 g fat, 0 g protein) 10 mL double cream (186 kJ, 0 g carbohydrate, 5 g fat, 0 g protein) 1 g unflavoured whey (17 kJ, 0 g carbohydrate, 0 g fat, 1 g protein)	216 mL orange juice (354 kJ, 19 g carbohydrate, 1 g fat, 1 g protein)	2917 kJ, 112 g (60%) carbohydrate, 19 g (25%) fat, 26 g (15%) protein

Table 2. Absolute and relative contributions of carbohydrate and fat oxidation in the high fat and high carbohydrate trials.

	Pre-prandial		Postprandial		Exercise		Post-exercise	
	Carbohydrate oxidation, $g \cdot min^{-1}$ [%]	Fat oxidation, $g \cdot min^{-1}$ [%]	Carbohydrate oxidation, $g \cdot min^{-1}$ [%]	Fat oxidation, $g \cdot min^{-1}$ [%]	Carbohydrate oxidation, $g \cdot min^{-1}$ [%]	Fat oxidation, $g \cdot min^{-1}$ [%]	Carbohydrate oxidation, $g \cdot min^{-1}$ [%]	Fat oxidation, $g \cdot min^{-1}$ [%]
High fat	0.19 (0.08) [48.6 (13.4)]	0.09 (0.03) [51.4 (13.4)]	0.17 (0.07)* [34.9 ± (10.1)]*	0.13 (0.05) [65.1 (10.1)]*	0.69 (0.19)* [39.7 (10.2)]*	0.45 (0.08)* [60.3 (10.2)]*	0.12 (0.06)* [25.4 (12.5)]*	0.16 (0.05) [74.6 (12.5)]*
High carbohydrate	0.21 (0.11) [48.9 (17.1)]	0.10 (0.04) [51.1 (17.1)]	0.25 (0.11) [46.6 (15.9)]	0.12 (0.05) [53.4 [15.9)]	0.84 (0.24) [47.5 (10.7)]	0.40 (0.07) [52.5 (10.7)]	0.17 (0.10) [33.6 (18.4)]	0.15 (0.06) [66.4 (18.4)]

Values are mean (SD), N = 12. % is percentage of energy yield. * Significantly ($P < 0.05$) different to high carbohydrate condition.

Supplementary Table 1. Palatability perceptions after the high fat and high carbohydrate breakfasts

	Appeal, mm	Smell, mm	Taste, mm	Aftertaste, mm	Palatability, mm
High fat	32 (17)	31 (16)	36 (25)	47 (26)	35 (23)
High carbohydrate	38 (22)	35 (17)	34 (22)	56 (26)	37 (20)

Values are mean (SD), N = 12.

Supplementary Table 2. Macronutrient intakes at the *ad-libitum* meal in the high fat and high carbohydrate conditions.

	Carbohydrate, g [%]	Fat, g [%]	Protein, g [%]
High fat	155 (57) [48 (11)]	59 (29) [39 (11)]	42 (19) [13 (2)]
High carbohydrate	160 (62) [47 (15)]	68 (34) [40 (14)]	48 (20) [13 (3)]

Values are mean (SD), N = 12.

References

1. Matu J, Deighton K, Ispoglou T, Duckworth L. The effect of moderate versus severe simulated altitude on appetite, gut hormones, energy intake and substrate oxidation in men. Appetite. 2017;113:284-92.

2. Wasse LK, Sunderland C, King JA, Batterham RL, Stensel DJ. Influence of rest and exercise at a simulated altitude of 4,000 m on appetite, energy intake, and plasma concentrations of acylated ghrelin and peptide YY. J Appl Physiol. 2012;112:552-9.

3. Westerterp-Plantenga MS, Westerterp KR, Rubbens M, Verwegen CR, Richelet JP, Gardette B. Appetite at "high altitude" [Operation Everest III (Comex-'97)]: a simulated ascent of Mount Everest. J Appl Physiol. 1999;87:391-9.

4. Aeberli I, Erb A, Spliethoff K, Meier D, Gotze O, Fruhauf H, et al. Disturbed eating at high altitude: influence of food preferences, acute mountain sickness and satiation hormones. Eur J Nutr. 2013;52:625-35.

5. Major GC, Doucet E. Energy intake during a typical Himalayan trek. High Alt Med Biol. 2004;5(3):355-63.

6. Armellini F, Zamboni M, Robbi R, Todesco T, Bissoli L, Mino A, et al. The effects of high altitude trekking on body composition and resting metabolic rate. Horm Metab Res. 1997;29:458-61.

7. Rose MS, Houston CS, Fulco CS, Coates G, Sutton JR, Cymerman A. Operation Everest. II: Nutrition and body composition. J Appl Physiol. 1988;65:2545-51.

8. Pulfrey SM, Jones PJ. Energy expenditure and requirement while climbing above 6,000 m. J Appl Physiol. 1996;81:1306-11.

9. Sergi G, Imoscopi A, Sarti S, Perissinotto E, Coin A, Inelmen EM, et al. Changes in total body and limb composition and muscle strength after a 6-8 weeks sojourn at extreme altitude (5000-8000 m). J Sports Med Phys Fit. 2010;50:450-5.

10. Hoppeler H, Vogt M. Muscle tissue adaptations to hypoxia. J Exp Biol. 2001;204:3133-9.

11. Westerterp KR, Kayser B, Wouters L, Le Trong JL, Richalet JP. Energy balance at high altitude of 6,542 m. J Appl Physiol. 1994;77:862-6.

12. Foster-Schubert KE, Overduin J, Prudom CE, Liu J, Callahan HS, Gaylinn BD, et al. Acyl and Total Ghrelin Are Suppressed Strongly by Ingested Proteins, Weakly by Lipids, and Biphasically by Carbohydrates. J Clin Endoc Metab. 2008;93:1971-9.

13. El Khoury D, El-Rassi R, Azar S, Hwalla N. Postprandial ghrelin and PYY responses of male subjects on low carbohydrate meals to varied balancing proportions of proteins and fats. Eur J Nutr. 2010;49:493-500.

14. Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men. J Clin Endoc Metab. 2006;91:1477-83.

15. Porrini M, Crovetti R, Testolin G, Silva S. Evaluation of Satiety Sensations and Food Intake After Different Preloads. Appetite. 1995;25:17-30.

16. Stubbs RJ. Nutrition Society Medal Lecture. Appetite, feeding behaviour and energy balance in human subjects. Proc Nutr Soc. 1998;57:341-56.

17. Tome D. Protein, amino acids and the control of food intake. The British journal of nutrition. 2004;92:27-30.

18. Tannous dit El Khoury D, Obeid O, Azar ST, Hwalla N. Variations in postprandial ghrelin status following ingestion of high-carbohydrate, high-fat, and high-protein meals in males. Annals Nutr Metab. 2006;50:260-9.

19. Hopkins M, Gibbons C, Caudwell P, Blundell JE, Finlayson G. Differing effects of high-fat or high-carbohydrate meals on food hedonics in overweight and obese individuals. British J Nutr. 2016;115:1875-84.

20. Yang J, Brown MS, Liang G, Grishin NV, Goldstein JL. Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone. Cell. 2008;132:387-96.

21. Kojima M, Hamamoto A, Sato T. Ghrelin O-acyltransferase (GOAT), a specific enzyme that modifies ghrelin with a medium-chain fatty acid. J Biochem. 2016;160:189-94.

22. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature. 1999;402:656-60.

23. Fernandez G, Cabral A, Cornejo MP, De Francesco PN, Garcia-Romero G, Reynaldo M, et al. Des-Acyl Ghrelin Directly Targets the Arcuate Nucleus in a Ghrelin-Receptor Independent Manner and Impairs the Orexigenic Effect of Ghrelin. J Neuroendoc. 2016;28:12349.

24. Nishi Y, Hiejima H, Hosoda H, Kaiya H, Mori K, Fukue Y, et al. Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin. Endocrinology. 2005;146:2255-64.

25. Lemarie F, Beauchamp E, Legrand P, Rioux V. Revisiting the metabolism and physiological functions of caprylic acid (C8:0) with special focus on ghrelin octanoylation. Biochimie. 2016;120:40-8.

26. Ashitani J-i, Matsumoto N, Nakazato M. Effect of octanoic acid-rich formula on plasma ghrelin levels in cachectic patients with chronic respiratory disease. Nutr J. 2009;8(1):25.

27. Acheson KJ. Influence of autonomic nervous system on nutrient-induced thermogenesis in humans. Nutr. 1993;9(4):373-80.

28. Bailey DP, Smith LR, Chrismas BC, Taylor L, Stensel DJ, Deighton K, et al. Appetite and gut hormone responses to moderate-intensity continuous exercise versus high-intensity interval exercise, in normoxic and hypoxic conditions. Appetite. 2015;89:237-45.

29. Goodman J, Hassell K, Irwin D, Witkowski EH, Nuss R. The splenic syndrome in individuals with sickle cell trait. High Alt Med Biol. 2014;15(4):468-71.

30. Fenn WO, Rahn H, Otis AB. A theoretical study of the composition of the alveolar air at altitude. Am J Physiol. 1946;146:637-53.

31. Conkin J. PH2O and simulated hypobaric hypoxia. Aviat Sp Environ Med. 2011;82:1157-8.

32. Stevenson E, Williams C, Nute M, Swaile P, Tsui M. The effect of the glycemic index of an evening meal on the metabolic responses to a standard high glycemic index breakfast and subsequent exercise in men. Int J Sport Nutr Ex Metab. 2005;15:308-22.

33. Wolever TM, Jenkins DJ, Ocana AM, Rao VA, Collier GR. Second-meal effect: low-glycemic-index foods eaten at dinner improve subsequent breakfast glycemic response. Am J Clin Nutr. 1988;48:1041-7.

34. Mellor A, Bakker-Dyos J, Howard M, Boos C, Cooke M, Vincent E, et al. British Services Dhaulagiri Medical Research Expedition: A unique military/civilian research collaboration. J Royal Army Med Corps. In Press.

35. Borg GA. Psychophysical bases of perceived exertion. Med Sci Sports Exercise. 1982;14:377-81.

36. Roach RC, Bärtsch P, Oelz O, Hackett PH. The Lake Louise acute mountain sickness scoring system. . In: Sutton JR, Houston CS, Goates G, editors. Hypoxia and Molecular Medicine. Burlington, VT: Queens City Press 1993. pp. 272-4.

37. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000;24:38-48.

38. Stubbs RJ, Hughes DA, Johnstone AM, Rowley E, Reid C, Elia M, et al. The use of visual analogue scales to assess motivation to eat in human subjects: a review of their reliability and validity with an evaluation of new hand-held computerized systems for temporal tracking of appetite ratings. Brit J Nutr. 2000;84:405-15.

39. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. J Appl Physiol. 1983;55:628-34.

40. Jeukendrup AE, Wallis GA. Measurement of substrate oxidation during exercise by means of gas exchange measurements. Int J Sports Med. 2005;26:28-37.

41. Date Y. The Vagus Nerve and Ghrelin Function. In: Portelli J, Smolders I, editors. Central Functions of the Ghrelin Receptor. New York, NY: Springer New York; 2014. pp. 53-61.

42. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. J Appl Physiol. 1974;37:247-8.

43. Fawcett JK, Wynn V. Effects of posture on plasma volume and some blood constituents. J Clin Pathol. 1960;13:304-10.

44. Hosoda H, Doi K, Nagaya N, Okumura H, Nakagawa E, Enomoto M, et al. Optimum collection and storage conditions for ghrelin measurements: octanoyl modification of ghrelin is rapidly hydrolyzed to desacyl ghrelin in blood samples. Clin Chem. 2004;50:1077-80.

45. Hopkins WG. How to interpret changes in an athletic performance test. Sportscience. 2004; 8:1–7.

46. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods. 2007;39:175-91.

47. Monteleone P, Bencivenga R, Longobardi N, Serritella C, Maj M. Differential responses of circulating ghrelin to high-fat or high-carbohydrate meal in healthy women. J Clin Endoc Metab. 2003;88:5510-4.

48. Scherer T, Buettner C. Yin and Yang of hypothalamic insulin and leptin signaling in regulating white adipose tissue metabolism. Rev Endoc Metab Disord. 2011;12:235-43.

49. Leonetti F, Iacobellis G, Ribaudo MC, Zappaterreno A, Tiberti C, Iannucci CV, et al. Acute insulin infusion decreases plasma ghrelin levels in uncomplicated obesity. Regul Pept. 2004;122:179-83.

50. Saad MF, Bernaba B, Hwu CM, Jinagouda S, Fahmi S, Kogosov E, et al. Insulin regulates plasma ghrelin concentration. J Clin Endoc Metab. 2002;87:3997-4000.

51. Anderwald C, Brabant G, Bernroider E, Horn R, Brehm A, Waldhausl W, et al. Insulin-dependent modulation of plasma ghrelin and leptin concentrations is less pronounced in type 2 diabetic patients. Diabetes. 2003;52:1792-8.

52. Vestergaard ET, Hansen TK, Gormsen LC, Jakobsen P, Moller N, Christiansen JS, et al. Constant intravenous ghrelin infusion in healthy young men: clinical pharmacokinetics and metabolic effects. Am J Physiol. 2007;292:1829-36.

53. Druce MR, Wren AM, Park AJ, Milton JE, Patterson M, Frost G, et al. Ghrelin increases food intake in obese as well as lean subjects. Int J Obes. 2005;29:1130-6.

54. Schmid DA, Held K, Ising M, Uhr M, Weikel JC, Steiger A. Ghrelin stimulates appetite, imagination of food, GH, ACTH, and cortisol, but does not affect leptin in normal controls. Neuropsychopharmacology. 2005;30:1187-92.

55. Wren AM, Seal LJ, Cohen MA, Brynes AE, Frost GS, Murphy KG, et al. Ghrelin enhances appetite and increases food intake in humans. J Clin Endoc Metab. 2001;86:5992.

56. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin increases energy intake in cancer patients with impaired appetite: acute, randomized, placebo-controlled trial. J Clin Endoc Metab. 2004;89:2832-6.

57. Babu AS, Veluswamy SK, Arena R, Guazzi M, Lavie CJ. Virgin coconut oil and its potential cardioprotective effects. Postgrad Med J. 2014 Nov;126:76-83.

58. Fukumori R, Sugino T, Shingu H, Moriya N, Kobayashi H, Hasegawa Y, et al. Ingestion of medium chain fatty acids by lactating dairy cows increases concentrations of plasma ghrelin. Domest Anim Endoc. 2013;45:216-23.

59. Zhang H, Li Y, Hou X, Zhang L, Wang T. Medium-chain TAG improve energy metabolism and mitochondrial biogenesis in the liver of intra-uterine growth-retarded and normal-birth-weight weanling piglets. Brit J Nutr. 2016;115:1521-30.

60. Blundell J. Pharmacological approaches to appetite suppression. Trends In Pharmacological Sciences. 1991;12:147-57.

61. Surina DM, Langhans W, Pauli R, Wenk C. Meal composition affects postprandial fatty acid oxidation. Am J Physiol. 1993;264:1065-70.

62. O'Hara JP, Woods DR, Mellor A, Boos C, Gallagher L, Tsakirides C, et al. A comparison of substrate oxidation during prolonged exercise in men at terrestrial altitude and normobaric normoxia following the coingestion of 13C glucose and 13C fructose. Physiol Rep. 2017; doi: 10.14814/phy2.13101

Figure Legends

Figure 1. Composite appetite score in the high fat (solid line) and high carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

Figure 2. Acylated ghrelin (A), des-acylated ghrelin (B) and total ghrelin (C) concentrations in the high fat (solid line) and high carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

Figure 3. Glucose (A), insulin (B) and lactate (C) concentrations in the high fat (solid line) and high carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.

Figure 4. Non-esterified fatty acids (A) and triglycerides (B) concentrations in the high fat (solid line) and high carbohydrate (dashed line) trials expressed as mean (SE), N = 12. Thin upward arrow represents breakfast and thick upward arrow represents *ad-libitum* meal. Black rectangle represents exercise.